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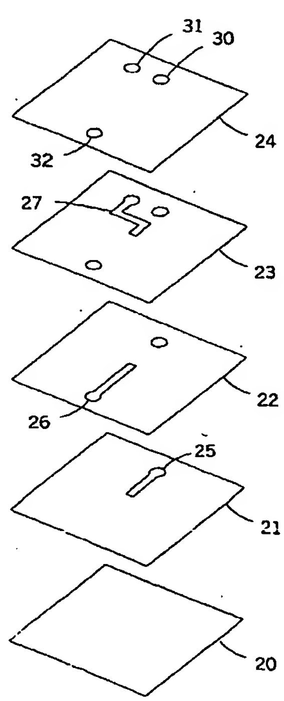
(54) Title: FLUIDIC MIXER IN MICROFLUIDIC SYSTEM



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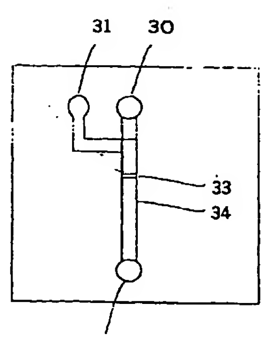

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(57) Abstract: Microfluidic devices capable of efficiently mixing one or more fluids are described. Two or more microfluidic channels within the device meet at an overlap region. The overlap region may be in fluid communication with an outlet channel. The inlet channels are disposed within different layers of a three dimension device. Microfluidic separators for separating multiphase fluids are also described. In this case, a multiphase fluid flows through an inlet channel into an overlap region from where the separated phases can be withdrawn through outlet channels. Also provided are methods for mixing and separating fluids in such devices.

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DESCRIPTION

Fluidic Mixer in Microfluidic System

Field Of The Invention

The present invention relates to microfluidic devices and the control of fluid flow within those devices. These devices are useful in various biological and chemical systems, as well as in combination with other liquid-distribution devices.

Background Of The Invention

There has been a growing interest in the manufacture and use of microfluidic systems for the acquisition of chemical and biological information. In particular, microfluidic systems allow complicated biochemical reactions to be carried out using very small volumes of liquid. These miniaturized systems increase the response time of the reactions, minimize sample volume, and lower reagent cost.

Traditionally, microfluidic systems have been constructed in a planar fashion using silicon fabrication techniques. Representative systems are described, for example, by Manz *et al.* (Trends in Anal. Chem. (1990) **10(5)**: 144-149; Advances in Chromatography (1993) **33**: 1-66). These publications describe microfluidic devices constructed using photolithography to define channels on silicon or glass substrates and etching techniques to remove material from the substrate to form the channels. A cover plate is bonded to the top of this device to provide closure.

More recently, a number of methods have been developed that allow microfluidic devices to be constructed from plastic, silicone or other polymeric materials. In one such method, a negative mold is first constructed, and plastic or silicone is then poured into or over the mold. The mold can be constructed using a silicon wafer (see, e.g., Duffy *et al.*, Analytical Chemistry (1998) **70**: 4974-4984; McCormick *et al.*, Analytical Chemistry (1997) **69**: 2626-2630), or by building a traditional injection molding cavity for plastic devices. Some molding facilities have developed techniques to construct extremely small molds. Components constructed using a lithography, electroplating and molding (LIGA)

technique have been developed (see, e.g., Schomburg *et al.*, Journal of Micromechanical Microengineering (1994) **4**: 186-191). Other approaches combine LIGA and a hot-embossing technique. Imprinting methods in polymethylinethacrylate (PMMA) have also been demonstrated (see, Martynova *et al.*, Analytical Chemistry (1997) **69**: 4783-4789) However, these techniques do not lend themselves to rapid prototyping and manufacturing flexibility. Additionally, these techniques are limited to planar structures. Moreover, the tool-up costs for both of these techniques are quite high and can be cost-prohibitive.

Generally, the mixing of fluids in a microfluidic system is problematic, since the fluid flow within these devices is not turbulent. Some microfluidic mixing devices have been constructed in substantially planar microfluidic systems where the fluids are allowed to mix through diffusion (see Bokenkamp *et al.*, Analytical Chemistry (1998) **70(2)**: 23 2-236. In these systems, the fluids only mix at the interface of the fluids, which is commonly small relative to the overall volume of the fluids. Thus, very little mixing occurs.

Alternative mixing methods have been developed based on electrokinetic flow. Such devices are complicated, requiring electrical contacts within the system. Additionally such systems only work with charged fluids, or fluids containing electrolytes. Finally, these systems require voltages that are sufficiently large that water is electrolyzed, which means bubble formation is a problem and samples can not be easily collected without being destroyed.

There is, thus, a need for a robust mixing device capable of thoroughly mixing a wide variety of fluids in a microfluidic environment in a controlled manner at relatively high speed.

Summary Of The Invention

One object of the present invention is to provide an inexpensive and robust microfluidic device that can control the mixing of two or more fluids.

An additional object of the present invention is to provide a microfluidic mixing device that can accommodate the use of a vast array of liquid reagents or solutions. Different types of solvents and samples can be mixed, including but not limited to water-based systems, organic-based systems, biological materials solvated or dispersed within solvent, chemical systems, and others known by those skilled in the art. The microfluidic devices of the present invention are constructed using a combination of traditional manufacturing techniques and novel chemistry and alignment procedures.

Microfluidic mixing devices are provided that have at least two inlet channels on different substantially planar layers of the device. The layers are horizontally disposed, such that a channel is substantially in a horizontal plane, and a channel in an adjacent layer can be above or below the first channel. The layers containing the inlet channels can be adjacent or can be separated by one or more layers. The inlet channels meet at an overlap region. Where the inlet channels are separated by more than one intervening layer, apertures in the intervening layers can extend the inlet channel to form the overlap region. An outlet channel is provided that is in fluid communication with the overlap region, such that fluids introduced from the inlet channels must proceed into the outlet channel.

Channels have at least one dimension less than about 500 microns. Channels also have an aspect ratio that maximizes surface to surface contact between fluid streams. A channel of the invention can have a depth from about I to about 500 microns, preferably from about 10 to about 100 microns, and a width of about 10 to about 10,000 microns such that the aspect ratio (width/depth) of the channel cross section is at least about 2, preferably at least about 10, at the overlap region where the channels meet.

The two or more inlet channels are in fluid communication at an overlap region. The overlap region is also in fluid communication with an outlet channel. The outlet channel can be on the same layer as one of the inlet channels or can be on a different layer. In a preferred embodiment, the outlet channel is on a layer intermediate between the inlet channels.

Definitions

The term "channel" as used herein is to be interpreted in a broad sense. Thus, it is not intended to be restricted to elongated configurations where the transverse or longitudinal dimension greatly exceeds the diameter or cross-sectional dimension. Rather, such terms are meant to comprise cavities or tunnels of any desired shape or configuration through which liquids may be directed. Such a fluid cavity may, for example, comprise a flow-through cell where fluid is to be continually passed or, alternatively, a chamber for holding a specified, discrete amount of fluid for a specified amount of time. "Channels" may be filled or may contain internal structures comprising valves or equivalent components.

The term "microfluidic"' as used herein is to be understood, without any restriction thereto, to refer to structures or devices through which fluid(s) are capable of being passed or directed, wherein one or more of the dimensions is less than 500 microns.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a figure of a basic microfluidic mixing device.

Figure 2 is a schematic of various ways to control the microfluidic mixing.

Figure 3 is a schematic of a microfluidic mixing device next to three prior art devices where minimal mixing occurs.

Figure 4 is a photograph of a prior art microfluidic mixing device where mixing does not occur, and a mixing device where complete mixing occurs.

Figure 5 is a schematic of a microfluidic mixer where three separate fluids can be mixed.

Figure 6 is a schematic of a combinatorial microfluidic mixer system.

Figure 7 is a microfluidic mixer constructed from etched silicon devices.

DETAILED DESCRIPTION OF THE INVENTION

The invention is directed to microfluidic mixing devices that provide rapid mixing of two or more fluids and includes microfluidic systems that are capable of mixing various fluids in a controlled manner based on the design and construction of the devices. In one embodiment, these devices contain microfluidic channels that are formed in various layers of a three dimensional structure. The various channels intersect in certain areas in order to produce mixing of the fluids in the various channels. The amount of overlap, geometry of the overlaps, surface chemistry of the overlaps, fluid used and flow rate of the fluids all have a controllable effect on the amount of mixing.

A microfluidic device of the invention has at least two inlet channels on different substantially planar, horizontally disposed, layers of the device. Such layers can be flexible, such that the overall device does not lie in a plane. The layers containing the inlet channels can be adjacent or can be separated by one or more layers. The inlet channels meet at an overlap region. Where the layers are stencil layers, and the channels are cut through the layers, the inlet channels must not overlap vertically until the overlap region, unless an intermediate layer is used. An outlet channel may be provided that is in fluid communication with the overlap region, such that fluid flowing through the inlet channels must enter the overlap region and exit through the outlet channel.

The inlet channels are in fluid communication at the overlap region. The overlap region is also in fluid communication with an outlet channel, if an outlet channel is provided. The outlet channel can be on the same layer as one of the inlet channels or can be on a different layer. In a preferred embodiment, the outlet channel is on a layer intermediate between the inlet channels.

This design produces sufficient interface per cross-sectional area between the different fluid streams to affect rapid mixing. In this manner, diffusional mixing is achieved between two or more fluids that meet at the overlap region, and they can mix to a greater degree than is usual in a microfluidic device. The shape and the amount of overlap at those points can be controlled in order to alter the amount of mixing.

In a preferred embodiment, the present invention is a microfluidic device comprising a plurality of microfluidic inlet channels and an overlap region within which said inlet channels are in fluid communication with each other. In a particular embodiment, at least one inlet channel is formed in a first sheet of a first material and at least another inlet channel is formed in a second sheet of a second material. The materials of the sheets may or may not be the same. For example, one sheet may be made of hydrophobic materials whereas the other sheet may be made of hydrophilic materials. The sheets may be made of materials that expedite fluid flow through the channels. Thus, the material of individual sheets may be selected depending on the composition and chemical nature of the fluid flowing through individual channels.

In another embodiment, the invention is a microfluidic device comprising a first inlet channel which is substantially parallel to the top and the bottom surfaces of the first sheet, a second inlet channel which is substantially parallel to the top and the bottom surfaces of the second sheet, and an overlap region within which said first and second inlet channels are in fluid communication with each other. In this embodiment the microfluidic device can further comprise an outlet channel in fluid communication with the inlet channels through the overlap region. In one embodiment, the outlet channel is formed in the first sheet or the second sheet. In a further version of this embodiment the first sheet and the second sheet are joined together such that the plane of the joint is substantially parallel to the top and bottom surfaces of the sheets.

A further embodiment of the invention is a device wherein the outlet channel is formed in a third sheet of the material such that the outlet channel is in a plane that is substantially parallel to the top and bottom surfaces of the third sheet. Further, the first, second and third sheets are joined together such that the planes of the joints are substantially parallel to the top and bottom surfaces of the sheets. Alternatively, the third sheet is joined to both the first sheet and the second sheet.

In another embodiment, the invention is a microfluidic mixer comprising a first sheet having a first channel through which a first fluid flows, a second sheet having a second channel through which a second fluid flows, and an overlap region formed by the

first and second channels such that the first fluid and the second fluid enter the overlap region and mix therein. This embodiment may further comprise an outlet channel in fluid communication with the inlet channels through the overlap region. Such an outlet channel, for example, is formed in the first sheet or the second sheet. Alternatively, the outlet channel is formed by in a substantially flat third sheet of the material such that the outlet channel is in a plane that is substantially parallel to the top and bottom surfaces of the third sheet.

In another embodiment, the invention is a microfluidic mixer comprising a first channel through which a first fluid flows, a second channel through which a second fluid flows, and an overlap region formed by the first and second channels such that the first fluid and the second fluid enter the overlap region and mix therein to form a mixture of the first and second fluids. Such a mixer may further comprise an outlet channel connected to the overlap region such that the mixture from the overlap region may flows through the outlet channel.

The mixer may be such that the first and second fluids are substantially the same or may differ in one or more of their properties, such as, viscosities, temperatures, flow rates, compositions.

In another embodiment, the device has two or more microfluidic inlet channels that are located within different layers of a three-dimensional device. The inlet channels are designed such that the flows of the fluids overlap, with a membrane separating the fluids from each other, and the flows run substantially in the same direction. The inlet channels end at an overlap region. The combined fluid flow then continues into the outlet channel that begins at the same overlap region. This outlet channel is in a layer between the two inlet channels, and is designed such that the direction of the resulting combined fluid flows in the same direction as the inlet fluids. Alternatively, the outlet channel can simply be an extension of one of the inlet channels.

In certain embodiments, a microfluidic device contains one or more of these fluidic overlaps. In certain embodiments, all of the fluidic mixers are identical. In other embodiments, the mixers differ within a single device in order to produce preferential

mixing. In certain embodiments, the mixers are multiplexed within a device to perform various applications. In another embodiment, the mixers are multiplexed within a device to create the possibility for combinatorial. synthesis of various types of materials.

The microfluidic devices of the invention also act as phase separators. A microfluidic device is constructed that has one inlet channel. The inlet channel terminates in an overlap region. A channel in the layer above the inlet channel and a channel in the layer below the inlet channel overlap the termination of the inlet channel as described above in the mixer embodiment. In use, a fluid enters the inlet area. Phase separation may occur in the inlet channel so that at the outlet channels two phases are separated into the two outlet channels. Examples of phase separation could be from oil/water mixtures where the oil rises to the top half of the inlet region and the water goes to the bottom portion. The oil then is withdrawn through the top inlet channel and the water exits out the bottom channel, resulting in separated phase streams.

The top portion of the inlet channel can be constructed from a different polymer than the bottom portion so as to expedite the phase separation. For example, in an organic/aqueous phase separation, the top half could be constructed from a hydrophobic material and the bottom half from a hyderphilic material. In use, the fluid within the channel will rearrange with the organic portion in the top half near the hydrophobic region and the aqueous portion in the bottom half near the hydrophilic region. The exit channels can be made of different materials as well to enhance this phase separation.

Thus, in a preferred embodiment, the invention may be a microfluidic separator comprising a first channel formed by removing a volume of material equal to the volume of the first channel from a substantially flat first sheet of the material such that the channel is substantially parallel to the top and the bottom surfaces of the first sheet, a second channel formed by removing a volume of material equal to the volume of the second channel from a substantially flat second sheet of the material such that the channel is substantially parallel to the top and the bottom surfaces of the second sheet, a third channel formed by removing a volume of material equal to the volume of the third channel from a substantially flat third sheet of the material such that the channel is substantially parallel to

the top and the bottom surfaces of the third sheet, and an overlap region within which said first, second and third inlet channels are in fluid communication with each other. In one version of this embodiment, the first sheet is sandwiched between the second and third sheets whereby the second channel is in fluid communication with the top half of the overlap region and the third channel is in fluid communication with the bottom half of the overlap region. In such a device, a fluid comprising a two-phase mixture is fed to the first channel under conditions sufficient to separate the two phases with the separated phases entering the overlap region such that one of the separated phases is withdrawn through the second channel and another of the separated phases is withdrawn through the third channel.

This embodiment may be such that the materials of the first, second and third sheets are substantially the same. Further, the materials of the second and third sheets can be selected such that the separation of the phases is expedited. For example, in one embodiment, the materials of the of the second and third sheets are selected such that the material of one of the sheets is hydrophobic and the material of another of the sheets is hydrophilic.

In another embodiment the above separator can be used in a method for separating phases of a multi-phase mixture. The method comprises the steps of feeding a fluid comprising a multi-phase mixture to the first channel under conditions sufficient to separate the two phases such that the separated phases enter the overlap region, withdrawing one of the separated phases through the second channel, and withdrawing another of the separated phases through the third channel.

In another embodiment, the invention is a method of manufacturing a microfluidic mixing device comprising the steps of removing a volume of material equal to the volume of a first channel from a substantially flat sheet of the material such that the channel is substantially parallel to the top and bottom surfaces of the sheet, removing a volume of material equal to the volume of a second channel from a substantially flat sheet of the material such that the channel is substantially parallel to the top and bottom surfaces of the

sheet, and forming an overlap region within which said first and second channels are in fluid communication with each other.

In still another embodiment, the invention is a method for mixing two or more fluids comprising transporting a first fluid at a first flow rate and a second fluid at a second flow rate through said first and second inlet channels of the various microfluidic devices described above. This method may be implemented under conditions such that the flow rates are substantially the same. Alternatively, the flow rates are controlled to change the composition in the overlap region of the devices described above.

Each of the inlet and outlet channels of the above devices and mixers are formed, for example, by removing a volume of material equal to the volume of the channel from a substantially flat sheet of the material. Examples of methods for removing the materials are set forth in co-pending applications, United States Patent Application Serial Nos. 09/550,184 and 09/453,029, the entire contents of which are herein incorporated by reference.

Examples of the microfluidic devices of the invention are devices wherein the volume of the inlet channels is between about I nanoliter to about 50 microliters per centimeter length of the inlet channel. The inlet channels have a rectangular or a square cross section with the length of each side between about 1 and about 500 microns. Alternatively, the inlet channels have a circular cross section with the diameter of the inlet channels between about 10 microns to about 1000 microns. Generally, channels have at least one dimension less than about 500 microns. Channels also have an aspect ratio that maximizes surface to surface contact between fluid streams. A channel of the invention can have a depth from about 1 to about 500 microns, preferably from about 10 to about 100 microns, and a width of about 10 to about 10,000 microns such that the aspect ratio (width/depth) of the channel cross section is at least about 2, preferably at least about 10, at the overlap region where the channels meet. A channel can be molded into a layer, etched into a layer, or can be cut through a layer. Where a channel is cut through a layer, the layer is referred to as a stencil layer.

Importantly, the nature of these microfluidic mixers is tuned for particular applications. Some of the parameters that affect the design of these systems include the type of fluid to be used, flow rate, and material composition of the devices. The microfluidic mixers described in the present invention can be constructed in a microfluidic device by controlling the geometry and chemistry of junction points.

Other microfluidic mixing devices have fluidic channels on a single substantially planar layer of a microfluidic device. Typically, the aspect ratio of these channels is 10:1 or greater. Such a constraint is in part a limitation of the silicon fabrication techniques used to produce such devices. The width of the channels is typically 10 to 500 times greater than the height of the channels. In order to mix samples, two coplanar inlet channels are brought together into a common outlet channel. The fluids meet at the intersection and proceed down the outlet channel. In microfluidic systems, all fluid flow is laminar (no turbulent flow occurs); thus, any mixing in this outlet channel occurs through diffusional mixing at the interface between the inputted liquid streams. This mixing is extremely slow since the interface between the two intersecting fluids is along the smaller dimensions of the perpendicular cross-sections of the fluid streams and is very small compared to the overall volume of the fluids. Since in these traditional microfluidic systems all channels are contained within the same substantially planar layer of the device, this problem is difficult to overcome. A microfluidic device approximating the prior art was constructed and is shown in Figure 3 and Figure 7.

In this invention, fluidic channels are located on different vertical layers of a three-dimensional device. When the channels are brought together to intersect into a common channel, the interface between the two fluids is along the horizontal dimension of the channels, which is the larger dimension of the perpendicular cross-section of the fluid streams. The larger interface maximizes the diffusion area between the fluids. In this manner, the majority of the volume of the fluids is in very close proximity to the diffusion interface of the mixing fluids and mixing occurs very rapidly. Importantly, the nature of these overlap regions must be carefully controlled in order to optimize the mixing, as will be described below.

In certain embodiments, the overlap region comprises one or more sheets containing apertures in fluid communication with the inlet channels.

In certain embodiments, the devices may further comprise an upper sheet, wherein said upper sheet provides the top surface of one inlet channel. Further, the devices may comprise a lower sheet, wherein said lower sheet provides the bottom surface of another inlet channel. These upper and lower sheets in certain embodiments are substantially rigid.

The material of the microfluidic devices of the invention is, for example, is paper, foil or a plastic. Examples of plastics are plastics selected from the group consisting of polytetrafluoroethylenes, polycarbonates, polypropylenes, polyimide and polyesters. Thus, the sheets of the invention can be made of plastic, paper or foil. The sheets of the invention can be joined together such that the plane of the joint is substantially parallel to the top and bottom surfaces of the sheets. In one embodiment, the first, second and third sheets are joined together such that the planes of the joints are substantially parallel to the top and bottom surfaces of the sheets. As an alternative, the third sheet is joined to both the first sheet and the second sheet such that it is sandwiched between the first and the second sheet.

In a preferred embodiment, these devices are constructed using stencil layers to define channels and/or chambers. A stencil layer is substantially planar and has a channel cut through it, such that in the final device, the top and bottom surfaces of the microfluidic channel within the stencil layer are formed from the bottom and top, respectively, of adjacent stencil or substrate layers. The stencils are preferably sandwiched between substrates, wherein the substrates are preferably substantially planar. Stencil layers are bonded by any technique that results in substantially liquid-tight channels within the device. The stencil can, for example, be self-adhesive to form a seal between adjacent substrates. Alternatively, an adhesive coating can be applied to the stencil layers. Alternatively, the stencil layers may be held together using gaskets and/or mechanical force. Alternatively, applying heat, light or pressure can activate adhesion. The construction of microfluidic devices from stencil layers and substrates is described in

co-pending applications, United States Patent Application Serial Nos. 09/550,184 and 09/453,029 the entire contents of which are herein incorporated by reference.

In one embodiment, the stencil layers are comprised of single- or double-sided adhesive tape. A portion of the tape (of the desired shape and dimensions) can be cut and removed to form channels, chambers and apertures. The tape stencil can then be placed on a supporting substrate or between layers of tape. In one embodiment, stencil layers can be stacked on each other. In this embodiment, the thickness or height of the channels can be varied by simply varying the thickness of the stencil (e.g., the tape carrier and the adhesive or glue thereon) or by using multiple identical stencil layers stacked on top of one another.

Various types of tape are useful in the above embodiment. Single- or double-sided adhesive tape is preferred. The type of glue or adhesive can be varied to accommodate the application, as can the underlying carrier's thickness and composition. Such tapes may have various methods of curing, including pressure sensitive tapes, temperature-curing tapes, chemically-curing tapes, optical-curing tapes, and other types of curing tapes. Examples include tapes that use rubber-based adhesive, acrylic-based adhesive, and other types of adhesive. The materials used to carry the adhesive are also numerous. Examples of suitable tape carrier materials include polyesters, polycarbonates, polytetrafluoroethylenes, polypropylenes, polyimides (e.g., KAPTONT™) and polyesters (e.g., MYLAR™). The thickness of these carriers can be varied.

In another embodiment, the layers are formed from silicon or similar materials, with channels etched therein. In such an embodiment, a set of channels is etched or created into the top surface of a silicon or glass substrate. A second set of channels is etched or created into a second substrate. The two substrates are adhered together in such a way that the channel surfaces are facing one another and certain regions are overlapping to form the mixing area. Such a device is described *infra.*

In yet another embodiment, the layers are not discrete, but a layer describes a substantially planar section through such a device. Such a device can be constructed using

photopolymerization techniques such as those described in Cumpston B.H. *et al.* (1999) *Nature* 398:51-54.

Example Devices

In the embodiment shown in Figure 1, a microfluidic mixing device is constructed by sandwiching stencil layers on top of one another. Referring to Figure IA, a microfluidic mixer is constructed by sandwiching four stencil layers 21-24 and adhering them to a substrate 20. The stencils have various channels 25-27 and apertures. Inlet ports 30, 31 and an outlet port 32 are in the top stencil layer 24. The assembled device is shown in Figure 1B. In use, a first fluid is injected into inlet port 30, passes through through-holes in layers 23 and 22 and enters channel 25. A second fluid enters entry port 31 and passes through channel 27. The two fluids meet at the overlap region 33 shown in Figure 1B. At this point, the fluids are forced to converge into a single channel 26 located in stencil layer 22. As the fluids meet and pass into channel 26, the top half of the channel is fluid two and the bottom half is fluid one. The height of these channels is relatively small (between 100 nm and 500 microns), so diffusional mixing quickly occurs and a homogenous material is transported off board at exit port 32. It has been discovered that the majority of the mixing occurs right at the junction point 33, with a slight amount of mixing occurring within channel 26 immediately after the junction point 34. The amount that mixing occurs after the junction point 33 depends on a number of factors, including geometry of the channels, chemical make-up of the channels and samples, flow rate, etc.

In the embodiment shown in Figure 1, the three channels that converge at point 32 are all the same width. Surprisingly, it has been discovered that if the layers containing the channels are not well aligned, proper mixing does not occur. The fluid entering outlet channel 26 is a mixture of the two input fluids only at points where channels 25, 26 and 27 all overlap. If, for example, inlet channel 25 is misaligned laterally such that for a small portion of the overlap there is an area where only inlet channel 27 and outlet channel 26 overlap, then in this region only the fluid from inlet channel 27 will enter outlet channel 26. The remainder of the fluid entering outlet channel 26 will be a mixture of the two input fluids; this will cause a "streaking" effect, where a flow of mixed fluids runs parallel

with an unmixed fluid through the outlet channel 26. These "streaking" problems are easily overcome by the following modifications.

Preferred mixer embodiments are shown in Figure 2. These embodiments do not suffer from the same strict alignment parameters that the mixer shown in Figure 1 has. Referring to Figure 2A, three different microfluidic mixers are built into a single device. The device is constructed from five stencil layers 40-44. Stencil layers have channels 45-53, through holes, entry ports 54,55 and exit ports 56 removed. The stencil layers are adhered together to form the completed device, shown in Figure 2B. Notice that the shapes of the overlap regions in these mixer devices 60-62 are shaped so that slight misalignment of layers during construction will not have a great effect on fluid flow and mixing. It has been found that mixers such as in Figure 2 are far superior to the mixer shown in Figure 1, for the above outlined reason.

In another preferred embodiment, changing the chemical nature in the overlap region alters the overlap junction. This can be accomplished by forming the stencil from a different material, or altering the surface chemistry of this stencil layer. The surface chemistry can be altered in many ways, as one skilled in the art will realize. These methods of altering the surface chemistry include chemical derivatization as well as surface modification techniques such as plasma cleaning or chemical etching. The chemical derivatization is preferably chosen such that fluids flow through the channels and overlap region occurs smoothly and without bubble formation.

The above-described methods for altering the overlap junction within a microfluidic device can be used independently or in conjunction with one another. Other methods for altering the nature of the junction are also included, if not specifically stated.

One surprising aspect of the present invention is that the optimal parameters for a given overlap are greatly affected by the nature of the sample that is to be used within the device. It has been found that the optimal geometry for these overlaps changes depending upon the solution used.

The mixing between two or more fluid channels can be adjusted to give a tremendous range of different ratios. The main or easiest way to do this is to hold the flow rate of one channel constant, while adjusting the flow rate of the other channel. In this way, different mixture ratios are formed by virtue of different quantities of each liquid entering the mixing chamber/ overlap area in a given time period. Another method is to alter the size of the channels leading into the mixing region; this has the effect of changing the flow rate internally. This would be useful for arrays, where different ratios are desired without the hassle of using different external flow rates.

The mixing characteristics of a microfluidic mixer according to this patent were compared to a number of microfluidic mixers that are the current state of the art. Referring to Figure 3, a single device is constructed that contains four independent microfluidic devices. The device is constructed from five stencil layers 80-84 that have channels 85-90 and through holes 91 cut into them. The stencils are constructed from layers of single sided tape (3 mil polypropylene carrier with water based adhesive on one side) and the channels are all 45 mils wide. The bottom stencil 80 is an 1/4" thick block of acrylic. Inlet ports 92,93 and outlet ports 94,95 are placed in the upper most stencil layer. All of the holes are 60 mils in diameter. The stencils are adhered together to form the completed device, shown in Figure 3B.

In the prior art, microfluidic devices are constructed in a 2-dimensional fashion such as the completed devices on the right of Figure 3B 97-99. If two different fluids are injected into the 2 inlet ports 92,93 of device 99, the fluids travel down their independent channels and meet at the central section of channel 90. In the central region, all of the flow is laminar. The fluids travel down their respective sides of the central channel until they reach the outlet channel areas 100,101. Surprisingly, the fluid that entered into inlet port 93 exits almost completely out of exit channel 100 and exit port 95. The fluid that entered 92, exits out of channel area 101 and exit port 94 almost exclusively. The only mixing that occurred in the central area of channel 90 is through diffusional mixing at the relatively small interface of the liquids. Because the width of these channels (about 60 mils) is much greater than their height (about 4 mils), the interfacial contact area between the two fluids is very small and the molecules at the interface must diffuse up to 30 mils in

order for complete mixing to occur. At room temperature, diffusional motion is not sufficiently rapid for this mixing to occur.

The prior art microfluidic mixers can be improved slightly by making the channel longer and thereby extending the interfacial contact area between the two fluids as in device 97 and 98. In these embodiments, the length of the mixing region is extended. However, very little mixing occurs even in these devices. In addition, a slower flow-rate allows more time for the diffusion process to occur. However, this also results in incomplete mixing over any reasonable time period.

Alternatively, an embodiment 96 of the microfluidic mixing device of the invention is shown. In this device, inlet channels 85,87 were constructed on different layers of a three dimensional structure. The inlet channels are in fluid communication at overlap region 102 where the two fluids to be mixed are forced to enter into outlet channel 86, in this case located on a layer intermediate to the layers containing the two inlet channels. In this embodiment, at the overlap region the interfacial contact area between the two fluids extends all the way across the width of the channel and is 15 times greater than in the previous device 99. In addition, the average distance that the molecules need to diffuse in order for mixing to occur is now 2 mils, rather than 30 mils as in the previous device 99.

This mixing behavior was demonstrated by performing a simple acid-base reaction within the mixers. A 0.1M NaOH solution was injected into one inlet channel 85, and a 0.5M HC1 solution injected into the other inlet channel 87. The NaOH solution contained a small amount of bromo-phenol blue indicator (which is purple in basic solution, and yellow in acidic solution). Upon entering the mixing area of the device 102, the clear HC1 solution and dark-purple NaOH solution mixed and reacted completely as evidenced by the color change of the indicator to a deep golden color (i.e. the stronger acidic solution neutralized the weaker basic solution, and the resulting mixture was weakly acidic). The reaction was also performed with a 0.1 M HC1 solution mixing with a 0.2M NaOH solution, in which the indicator was first dissolved in the acidic solution. In this experiment, the clear NaOH solution and yellow HC1 solution mixed to create a dark

purple fluid (in this case, the weaker acid is neutralized by the stronger base, resulting in a mixture that is weakly basic). In comparison, a device of the prior art was also tested using these same solutions. In this device, little or no mixing occurred at the interface of the two liquids. The solutions that came out of the outlets on either side were the same color and pH as the solutions that were inputted at the corresponding inlet side.

The two fluids were then injected into the microfluidic mixer described in this invention. Again, a clear NaOH solution was input at inlet 92 and a yellow HCI solution (containing indicator) at inlet 93. The two fluids begin to mix at the overlap region 102 and the mixing is nearly complete just after this region 103. Dark fluid color was observed within the exit channel 86 and at the outlet ports 106,107, which was indicative of the acid-base reaction going to completion.

The mixing behavior was also demonstrated by injecting water that had been dyed yellow into entry port 93 and blue dyed fluid into entry port 92. As the fluid flowed down the prior art microfluldic channels, not mixing occurred. Referring to Figure 4A, yellow fluid injected into port 93 and blue fluid into entry port 92. Notice, no mixing occurs in the channel length. Referring to Figure 4B, fluids were injected into a snaking channel; still no mixing occurred. Finally, referring to Figure 4C, the colored fluids were injected into the microfluidic mixer described here. The two fluids begin to mix at the overlap region 102 and the mixing is complete just after this region 103 and all of the fluid is green.

More than two fluids can also be mixed with this invention. Referring to Figure 5, a microfluidic mixer that brings in three different fluids and mixes them is shown. Figure 5A shows seven stencil layers 120-126 that have channels 127-129 and through regions 130,131 and through holes 132. All of the channels are 60 mils wide and the holes are all 80 mils in diameter. The stencil layers are all constructed from single sided tape (3 mil thick polypropylene backing with water based adhesive). The bottom stencil 120 is a 1/4" thick block of acrylic. The top stencil layers 126 has inlet ports 133 and an exit port 134 in it. The assembled device is shown in Figure 5B.

In use, three different fluids are injected at the inlet ports 133. They each travel down their respective channels 127-129 and meet at the junction point 135. In this region, the fluid from channel 129 is forced into the top third of channel region 136, the fluid from 128 occupies the middle third of region 136 and the fluid from 127 occupies the bottom third of 136. Again, the interfacial contact area between the two fluids is now maximized in the channel area 136 between the three fluids and diffusional mixing occurs very rapidly, so that the fluid that exits port 134 is fully mixed. This device also allows for a tremendous range in the mixing ratios. The flow rates of each of the fluids can be adjusted to allow a greater or lesser amount of each fluid to be added to the mixture.

A combinatorial microfluidic mixer is shown in Figure 6. This device brings in two different fluids and mixes them in four different stoichiometries.

Referring to Figure 6A, a combinatorial microfluidic mixer is constructed from five stencil layers 150 - 154 that has channels 155-159 and through-holes 160,161. Inlet ports 162,163 and outlet ports 164-167 are located in the top stencil layer 154. Channels 157 and 158 are 45 mils wide, channels 155, 156 and 159 are 30 mils wide and the overlap regions are 15 mils. All of the round areas and holes are 70 mils in diameter. The stencil layers are all constructed from single sided tape (3 mil thick polypropylene backing with water based adhesive). The bottom stencil 150 is a 1/4" thick block of acrylic. In use, fluid A is injected at port 162 and fluid B at port 163. The fluids are split in the division sections of channel 158. At the microfluidic mixer region 168 fluids A and B mix to form A+B. The fluids move on to the splitter channels 157 to the next set of microfluidic; mixer regions. At region 169, the mixture of A+B meets with pure A. The output is 3A+B. At region 170, A+B meets with pure B, outputting 3B+A. The outputs of the combinatorial microfluidic mixer are as follows: 164 is pure B, 165 is 3B+A, 166 is 3A+B, 167 is pure A. Other combinations can be constructed. In practice the amounts of fluid mixing at each of the output is dependent on a number of factors, including flow rate, fluid properties and device geometry and chemistry.

A microfluidic device of the invention were constructed using silicon fabrication techniques. Referring to Figure 7A, a channel 181 is patterned in (110) Si substrate 180

using an oxide mask and etched in 70°C KOH. The channel 181 is etched so that it is 100 microns wide and 3 microns deep. A second channel 183 is similarly patterned and etched in another (110) Si substrate 182. Holes 184-186 are drilled all the way through the second substrate 182. These holes are 800 microns in diameter.

The two substrates 180 and 182 are aligned face-to-face and the two surface 187,188 are aniodically bonded to form a seal as shown in the composite drawing Figure 7B.

In use, two different fluids are injected at inlet ports 184 and 185. They each travel down their respective channels and meet at the junction point 189. Again, the interfacial contact area between the two fluids is maximized in the channel area 189 between the two fluids and diffusional mixing occurs very rapidly, so that once the fluid reached region 190 is it fully mixed.

The invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed, since these embodiments are intended merely to illustrate certain aspects of the invention. All equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosures of all references cited herein are incorporated by reference in their entireties.

Claims

1. A microfluidic device comprising:

a plurality of microfluidic inlet channels; and

an overlap region within which said inlet channels are in fluid communication with each other.

2. The microfluidic device of claim 1, wherein each of the inlet channels comprises a channel formed by removing from a substantially flat sheet of material a volume of siad material equal to the volume of the channel.

3. The microfluidic device of claim 2, wherein at least one inlet channel is formed in a first sheet of a first material and at least another inlet channel is formed in a second sheet of a second material.

4. The microfluidic device of claim 3, wherein the first material and the second material are substantially the same.

5. The microfluidic device of claim 3, wherein the first material and the second material are not the same.

6. The microfluidic device of claim 5, wherein the first material is hydrophobic and the second material is hydrophilic.

7. The microfluidic device of claim 1 wherein the volume of the inlet channels is between about I nanoliter to about 50 microliters per centimeter length of the inlet channel.

8. The microfluidic device of claim 1 wherein the inlet channels have a rectangular or a square cross section.

9. The microfluidic device of claim 8, wherein the length of each side is between about 1 and about 500 microns.

10. The microfluidic device of claim 1, wherein the inlet channels have a circular cross section.

11. The microfluidic device of claim 10, wherein the diameter of the inlet channels is between about 10 microns to about 1000 microns.

12. The microfluidic device of claim 2, wherein said material is paper, foil or a plastic.

13. The microfluidic device of claim 13, wherein said plastic is selected from the group consisting of polytetrafluoroethylenes, polycarbonates, polypropylenes, polyimide and polyesters.

14. A microfluidic device comprising:

a first inlet channel formed by removing a volume of material equal to the volume of the first inlet channel from a substantially flat first sheet of said material such that the channel is substantially parallel to the top and the bottom surfaces of the first sheet;

a second inlet channel formed by removing a volume of material equal to the volume of the second inlet channel from a substantially flat second sheet of the material such that the channel is substantially parallel to the top and the bottom surfaces of the second sheet; and

an overlap region within which said first and second inlet channels are in fluid communication with each other.

15. The microfluidic device of claim 14, further comprising an outlet channel in fluid communication with the inlet channels through the overlap region.

16. The microfluidic device of claim 15 wherein the volumes of the inlet channels and the outlet channel are between about I nanoliter to about 50 microliters per centimeter length of the inlet channel.

17. The microfluidic device of claim 15 wherein the inlet and outlet charmels have a rectangular or a square cross section.

18. The microfluidic device of claim 17, wherein the length of each side is between about 1 and about 500 microns.

19. The microfluidic device of claim 15, wherein the inlet and outlet channels have a circular cross section.

20. The microfluidic device of claim 19, wherein the diameter of the inlet channels is between about 10 microns to about 1000 microns.

21. The microfluidic device of claim 15, wherein said outlet channel is formed by removing a volume of material equal to the volume of the outlet channel from the first sheet or the second sheet.

22. The microfluidic device of claim 21, wherein the first sheet and the second sheet are joined together such that the plane of the joint is substantially parallel to the top and bottom surfaces of the sheets.

23. The microfluidic device of claim 15, wherein said outlet channel is formed by removing a volume of material equal to the volume of the outlet channel from a substantially flat third sheet of the material such that the outlet channel is in a plane that is substantially parallel to the top and bottom surfaces of the third sheet.

24. The microfluidic device of claim 23, wherein the first, second and third sheets are joined together such that the planes of the joints are substantially parallel to the top and bottom surfaces of the sheets.

25. The microfluidic device of claim 24, wherein the third sheet is joined to both the first sheet and the second sheet.

26. The microfluidic device of claim 14, wherein said overlap region comprises one or more sheets containing apertures in fluid communication with said first and second inlet channels.

27. The microfluidic device of claim 14, wherein said sheets are stencil sheets.

28. The microfluidic device of claim 14, further comprising an upper sheet, wherein said upper sheet provides the top surface of one inlet channel.

29. The microfluidic device of claim 14, further comprising a lower sheet, wherein said lower sheet provides the bottom surface of the other inlet channel.

30. The microfluidic device of claim 28, wherein said upper sheet is substantially rigid.

31. The microfluidic device of claim 29, wherein said lower sheet is substantially rigid.

32. The microfluidic device of claim 23, wherein the material of said sheets is paper, foil or plastic.

33. The microfluidic device of claim 32, wherein said sheets are adhesively bonded.

34. The microfluidic device of claim 32, wherein said sheets are self-adhesive having a carrier layer and an adhesive layer.

35. The microfluidic device of claim 34, wherein said carrier layers are selected from the group consisting of polytetrafluoroethylenes, polycarbonates, polypropylenes, polyimide and polyesters.

36. A microfluidic mixer comprising:

a first sheet having a first channel through which a first fluid flows;

a second sheet having a second channel through which a second fluid flows; and

an overlap region formed by the first and second channels such that the first fluid and the second fluid enter the overlap region and mix therein.

37. The microfluidic device of claim 36, further comprising an outlet channel in fluid communication with the inlet channels through the overlap region.

38. The microfluidic device of claim 37 wherein the volumes of the inlet channels and the outlet channel are between about 1 nanoliter to about 50 microliters per centimeter length of the inlet channel.

39. The microfluidic device of claim 37 wherein the inlet and outlet channels have a rectangular or a square cross section.

40. The microfluidic device of claim 39, wherein the length of each side is between about 1 and about 500 microns.

41. The microfluidic, device of claim 37, wherein the inlet and outlet channels have a circular cross section.

42. The microfluidic device of claim 41, wherein the diameter of the inlet channels is between about 10 microns to about 1000 microns.

43. The microfluidic device of claim 37, wherein said outlet channel is formed by removing a volume of material equal to the volume of the outlet channel from the first sheet or the second sheet.

44. The microfluidic device of claim 43, wherein the first sheet and the second sheet are joined together such that the plane of the joint is substantially parallel to the top and bottom surfaces of the sheets.

45. The microfluidic device of claim 37, wherein said outlet channel is formed by removing a volume of material equal to the volume of the outlet channel from a substantially flat third sheet of the material such that the outlet charmel is in a plane that is substantially parallel to the top and bottom surfaces of the third sheet.

46. The microfluidic device of claim 45, wherein the first, second and third sheets are joined together such that the planes of the joints are substantially parallel to the top and bottom surfaces of the sheets.

47. The microfluidic device of claim 46, wherein the third sheet is joined to both the first sheet and the second sheet.

48. The microfluidic device of claim 36, wherein said overlap region comprises one or more sheets containing apertures in fluid communication with said first and second inlet channels.

49. The microfluidic device of claim 36, wherein said sheets are stencil sheets.

50. The microfluidic device of claim 36, further comprising an upper sheet, wherein said upper sheet provides the top surface of one inlet channel.

51. The microfluidic device of claim 36, further comprising a lower sheet, wherein said lower sheet provides the bottom surface of the other inlet channel.

52. The microfluidic device of claim 50, wherein said upper sheet is substantially rigid.

53. The microfluidic device of claim 51, wherein said lower sheet is substantially rigid.

54. The microfluidic device of claim 45, wherein the material of said sheets is paper, foil or plastic.

55. The microfluidic device of claim 54, wherein said sheets are adhesively bonded.

56. The microfluidic device of claim 54, wherein said sheets are self-adhesive having a carrier layer and an adhesive layer.

57. The microfluidic device of claim 56, wherein said carrier layers are selected from the group consisting of polytetrafluoroethylenes, polycarbonates, polypropylenes, polyimide and polyesters.

58. A microfluidic mixer comprising:

a first channel through which a first fluid flows;

a second channel through which a second fluid flows; and

an overlap region formed by the first and second channels such that the first fluid and the second fluid enter the overlap region and mix therein to form a mixture of the first and second fluids.

59. The microfluidic mixer of claim 58, further comprising an outlet channel connected to the overlap region such that the mixture from the overlap region may flow through the outlet channel.

60. The microfluidic device of claim 59 wherein the volumes of the inlet channels and the outlet channel are between about I nanoliter to about 50 microliters per centimeter length of the inlet channel.

61. The microfluidic device of claim 59 wherein the inlet and outlet channels have a rectangular or a square cross section.

62. The microfluidic device of claim 61, wherein the length of each side is between about I and about 500 microns.

63. The microfluidic device of claim 59, wherein the inlet and outlet channels have a circular cross section.

64. The microfluidic device of claim 63, wherein the diameter of the inlet channels is between about 10 microns to about 1000 microns.

65. The microfluidic mixer of claim 59, wherein each of the channels is formed by removing a volume of material equal to the volume of said channel from substantially flat sheets of the material.

66. The microfluidic mixer of claim 65, wherein the sheets are joined together such that the plane of the joint is substantially parallel to the top and bottom surfaces of the sheets.

67. The microfluidic mixer of claim 65, wherein said outlet channel is formed by removing a volume of material equal to the volume of the outlet channel from a sheet that contains one of the inlet channels.

68. The microfluidic mixer of claim 65, wherein each of the first and second inlet channels is formed by removing a volume of material equal to the volume of said channel from substantially flat first and second sheets of the material and said outlet channel is formed by removing a volume of material equal to the volume of the outlet channel from a third sheet of the material.

69. The microfluidic mixer of claim 68, wherein the third sheet is sandwiched between the first and second sheets.

70. The microfluidic mixer of claim 58, wherein said overlap region comprises one or more sheets containing apertures in fluid communication with said first and second inlet channels.

71. The microfluidic mixer of claim 65, wherein said sheets are stencil sheets.

72. The microfluidic mixer of claim 68, further comprising an upper sheet, wherein said upper sheet provides the top surface of one inlet channel.

73. The microfluidic, mixer of claim 68, further comprising a lower sheet, wherein said lower sheet provides the bottom surface of the other inlet channel.

74. The microfluidic, mixer of claim 72, wherein said upper sheet is substantially rigid.

75. The microfluidic, mixer of claim 73, wherein said lower sheet is substantially rigid.

76. The microfluidic, mixer of claim 65, wherein the material of said sheets is paper, foil or plastic.

77. The microfluidic mixer of claim 76, wherein said sheets are adhesively bonded.

78. The microfluidic mixer of claim 76, wherein said sheets are self-adhesive having a carrier layer and an adhesive layer.

79. The microfluidic mixer of claim 78, wherein said carrier layers are selected from the group consisting of polytetrafluoroethylenes, polycarbonates, polypropylenes, polyimide and polyesters.

80. The microfluidic mixer of claim 58 wherein the first and second fluids are substantially the same.

81. The microfluidic mixer of claim 58 wherein the first and second fluids have different viscosities.

82. The microfluidic, mixer of claim 58 wherein the first and second fluids have different compositions.

83. The microfluidic mixer of claim 58 wherein the first and second fluids have different temperatures.

84. The microfluidic mixer of claim 58 wherein the first and second fluids have different flow rates.

85. A microfluidic separator for separating phases of a multi-phase fluid mixture comprising:

a first channel formed by removing a volume of material equal to the volume of the first channel from a substantially flat first sheet of the material such that the channel is substantially parallel to the top and the bottom surfaces of the first sheet;

a second channel formed by removing a volume of material equal to the volume of the second channel from a substantially flat second sheet of the

material such that the channel is substantially parallel to the top and the bottom surfaces of the second sheet;

a third channel formed by removing a volume of material equal to the volume of the third channel from a substantially flat third sheet of the material such that the channel is substantially parallel to the top and the bottom surfaces of the third sheet; and

an overlap region within which said first, second and third inlet channels are in fluid communication with each other.

86. The microfluidic device of claim 85, wherein the first sheet is sandwiched between the second and third sheets whereby the second channel is in fluid communication with the top half of the overlap region and the third channel is in fluid communication with the bottom half of the overlap region.

87. The microfluidic device of claim 85, wherein the materials of the first, second and third sheets are substantially the same.

88. The microfluidic device of claim 85, wherein the materials of the second and third sheets are selected such that the separation of the phases is expedited.

89. The microfluidic device of claim 85, wherein the materials of the of the second and third sheets are selected such that the material of one of the sheets is hydrophobic and the material of another of the sheets is hydrophilic.

90. A method for separating phases of a multi-phase mixture using the separator of claim 85, the method comprising the steps of:

feeding a fluid comprising a multi-phase mixture to the first channel under conditions sufficient to separate the phases such that the separated phases enter the overlap region;

withdrawing one of the separated phases through the second channel; and

withdrawing another of the separated phases through the third channel.

91. A method of manufacturing a microfluidic mixing device comprising:

removing a volume of material equal to the volume of a first channel from a substantially flat sheet of the material such that the channel is substantially parallel to the top and bottom surfaces of the sheet;

removing a volume of material equal to the volume of a second channel from a substantially flat sheet of the material such that the channel is substantially parallel to the top and bottom surfaces of the sheet; and

forming an overlap region within which said first and second channels are in fluid communication with each other.

92. A method for mixing two or more fluids comprising transporting a first fluid at a first flow rate and a second fluid at a second flow rate through said first and second inlet channels of the microfluidic device of claim 14.

93. The method of claim 92, wherein said flow rates are substantially the same.

94. The method of claim 92, wherein said flow rates are controlled to change the composition in the overlap region.

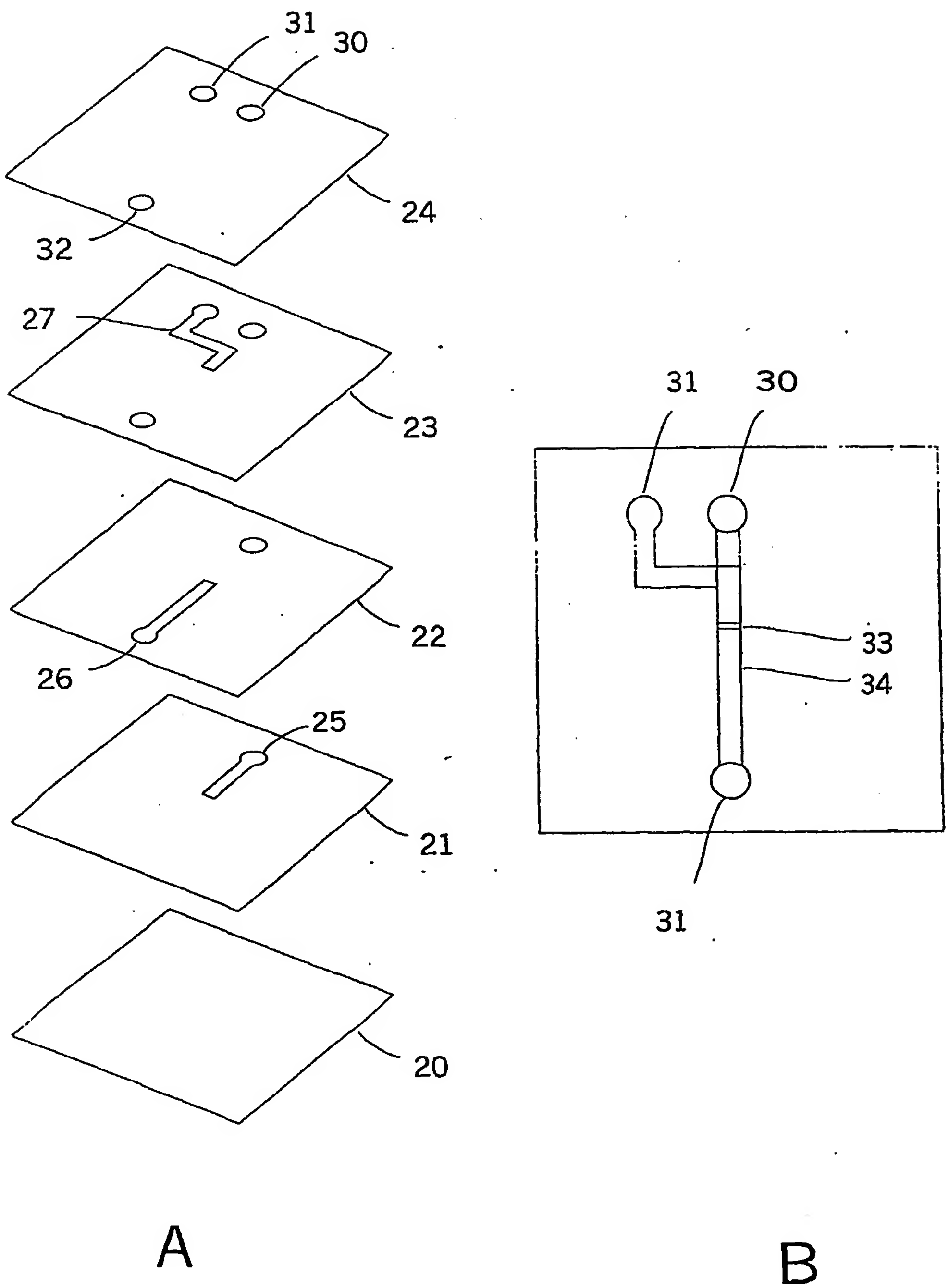
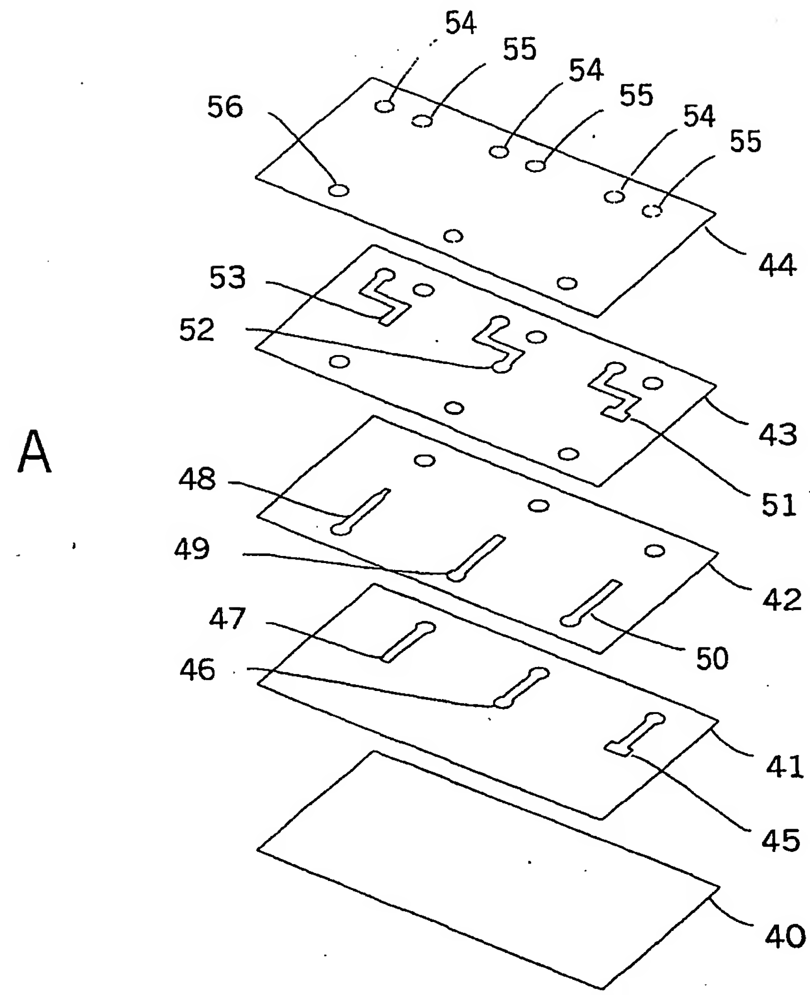


Figure 1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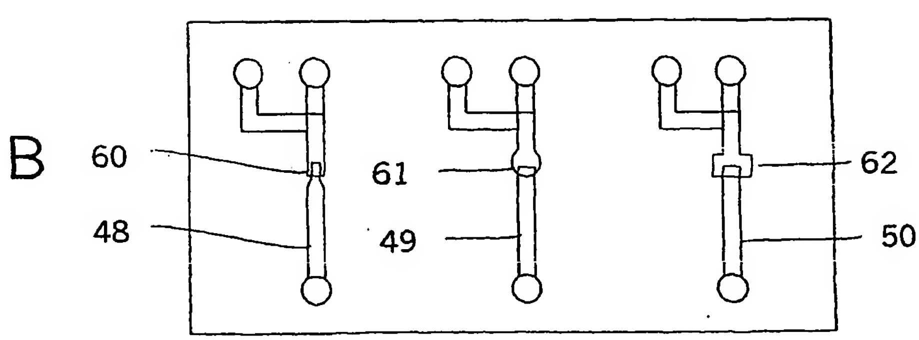
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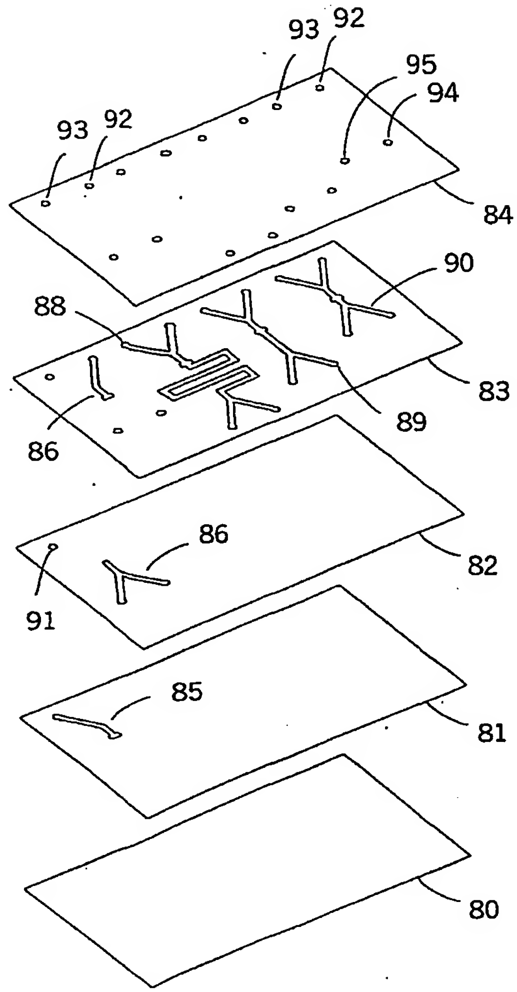
Figure 2



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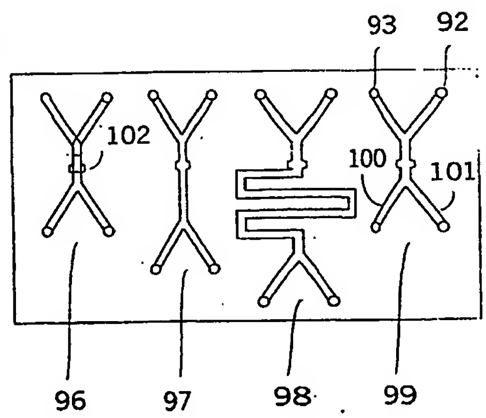

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Figure 3

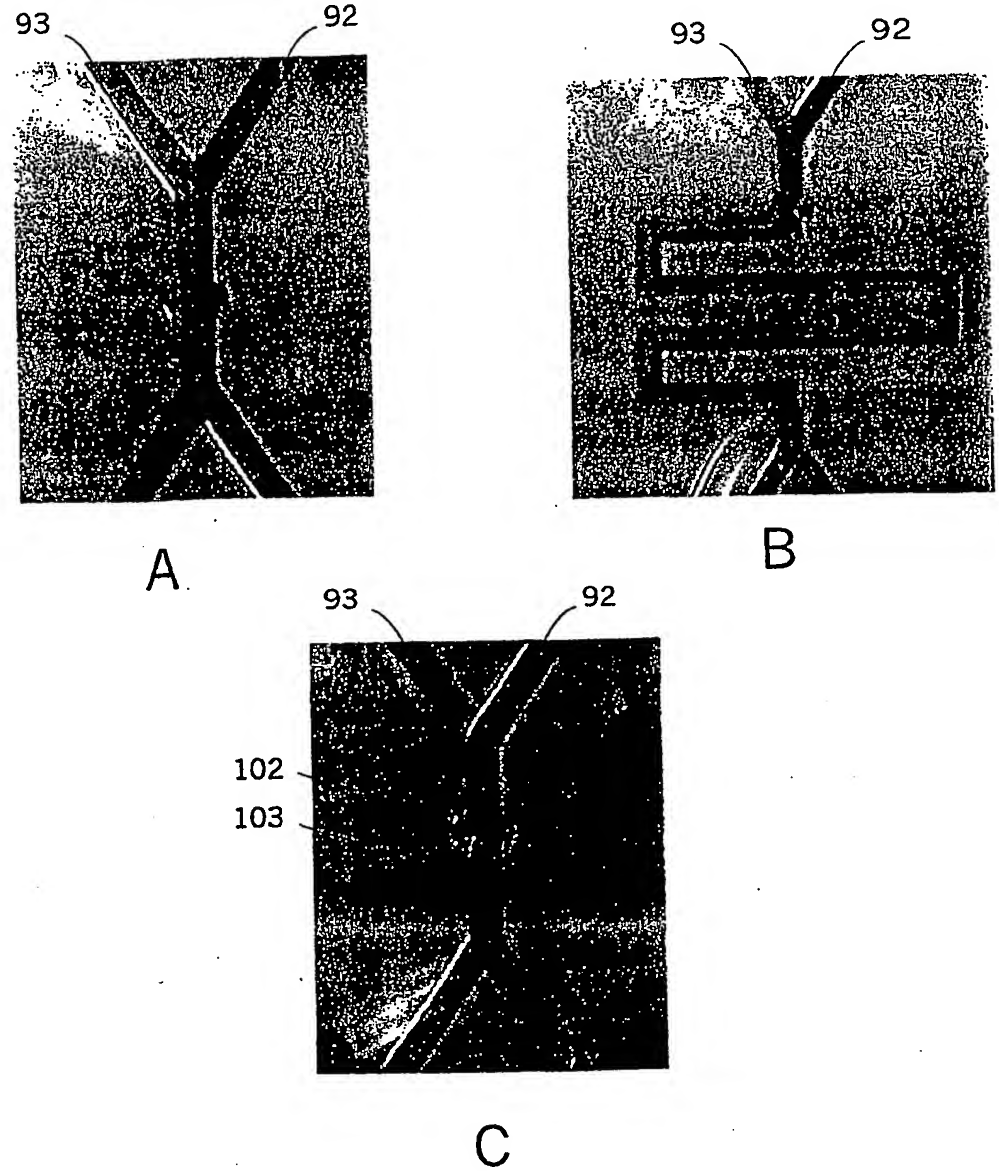

Figure 4

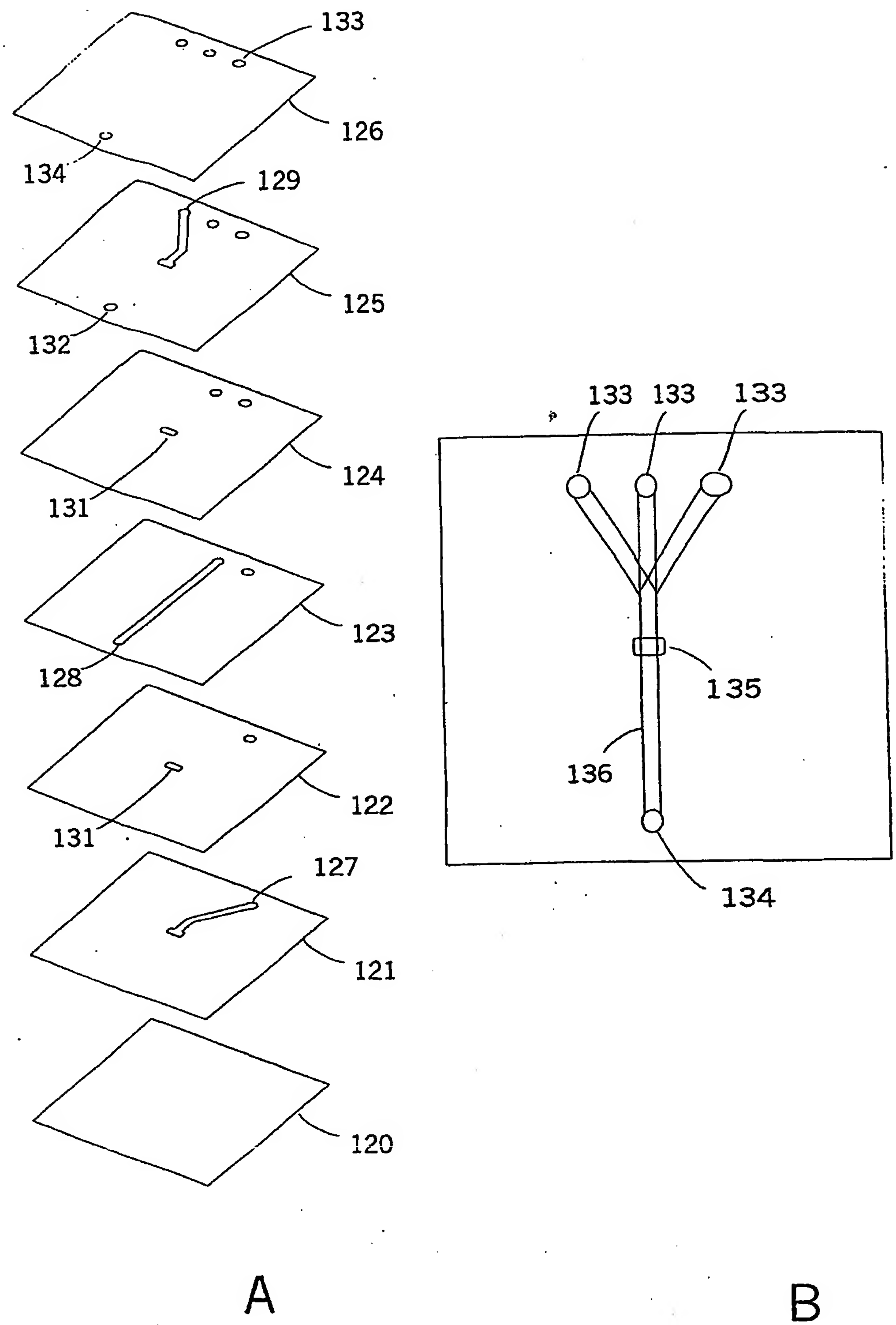
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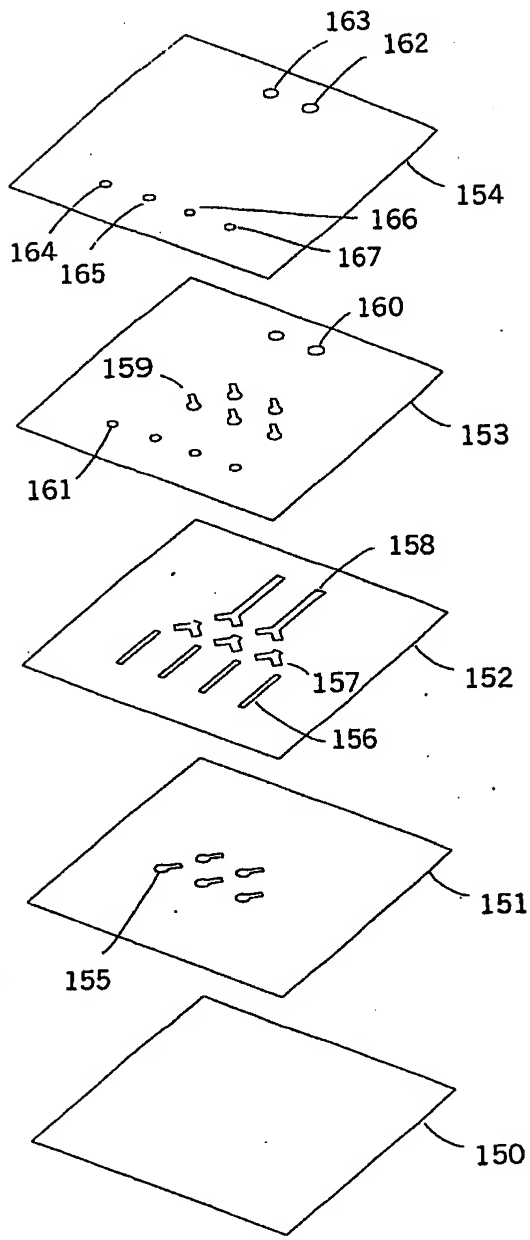
Figure 5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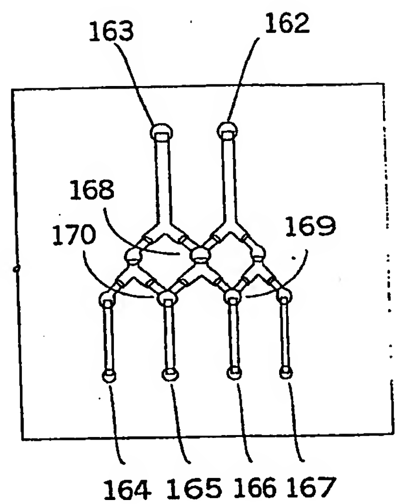
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Figure 6

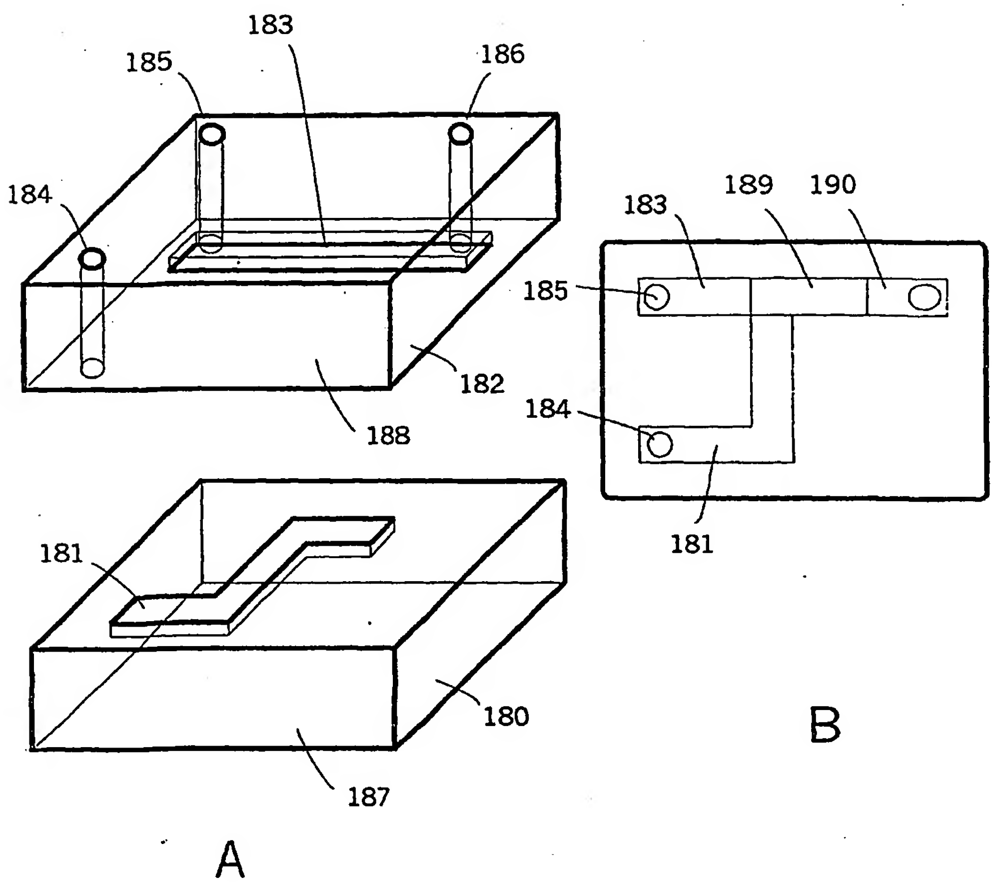

Figure 7